A STUDY OF BORRELIA ANSERINA INFECTION (SPIROCHETOSIS) IN TURKEYS[1]

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Although avian spirochetosis has been endemic in many parts of the world for several decades, it has only recently been positively identified in North America (Hoffman, Jackson, and Rucker, 1946; Hoffman and Jackson, 1946). Ward and Gallagher (1920) and Kaupp (1922) stated that some workers have suspected its presence in the United States, but no positive diagnoses were made. Burroughs (1947) reported the first finding of this spirochete in ticks in the United States when he discovered that specimens of *Argas persicus* sent to him from Texas were infected. The disease is caused by *Borrelia anserina* (*Spirochaeta anserina*), which was first described from geese in the Caucasus by Sakharoff in 1891. Marchoux and Salimbeni (1903) in Brazil were the first to report a natural outbreak in fowls and also to incriminate *Argas persicus* as the arthropod vector. Since then it has been reported as a major cause of mortality in fowl in many parts of the world. Recent reviews of the literature are given by Knowles, Das Gupta, and Basu (1932), Reis and Nobrega (1936), Sreenivasan and Sankaranarayan (1945), and El-Dardiry (1945). Knowles, Das Gupta, and Basu (1932) give an excellent review of 180 papers covering the period of 1891–1931. Stavitsky (1948) gives a good general discussion of the three genera of pathogenic spirochetes.

Since the disease has only recently been reported in this country, and since there are only casual references in the literature to its natural occurrence in turkeys previous to the accounts of Hoffman *et al.* (1946), it seemed advisable to conduct experiments with this strain in turkeys. This paper reports the results of these experiments as well as a historical discussion of the disease and studies on a second field outbreak in turkeys.

DESCRIPTION OF THE ORGANISM

Sakharoff (1891) first described the organism from the blood of geese suffering from a severe febrile disease in the Caucasus. His original description includes a photomicrograph which shows about six spirals, but does not give measurements of length. Reports in the literature of the length have varied from 6 to 30 μ, and there is wide variation in the same bird, due to division stages. Hinshaw and McNeil (1946) reported an average of 14 μ (7 to 21 μ) with six spirals (figure 1). The organism is motile, stains readily with aniline dyes (in contrast to *Leptospira* and *Treponema*), and is soluble in 10 per cent ox bile and 10 per cent saponin. At crisis the spirochetes are in large clumps and are often granu-

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lar (figure 2). *Borrelia anserina* also differs from *Treponema* in having loose, rather than tight, coils and in the ease with which it can be stained. It differs from *Leptospira* in having looser coils and in the absence of a terminal hook as well as in ease of staining. It is most easily diagnosed from blood smears but may also be found in sections of almost any tissue. In the tissues it is best demonstrated by some method of silver impregnation, such as that of Levaditi, or by the slow method of Giemsa.

Among its various synonyms are *Spirochaeta gallinarum*, *Treponema anserinum*, *Spirochaeta anserina*, and *Spirochaeta anatis*. The 1948 edition of *Bergey's*

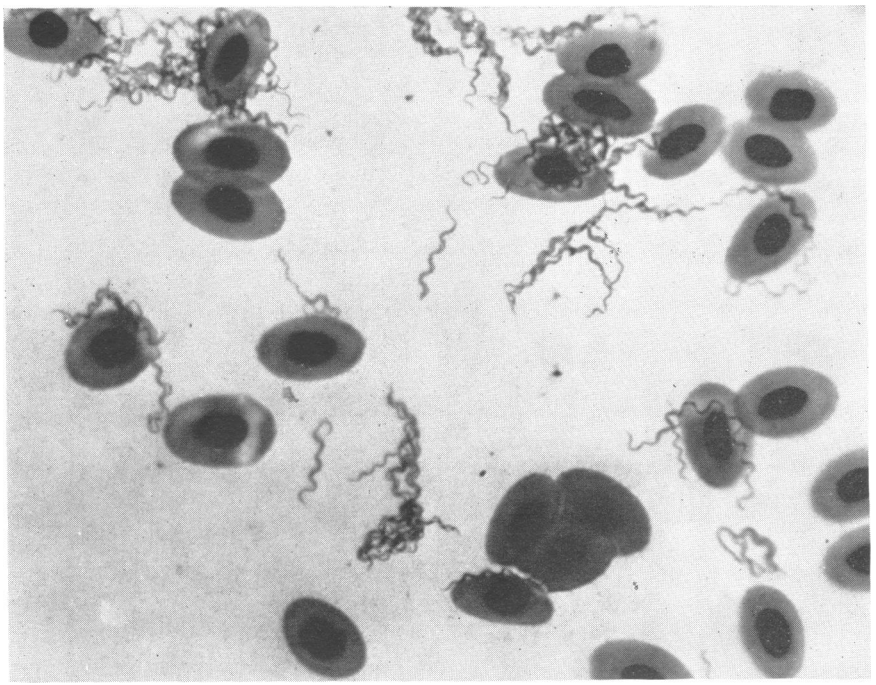

Figure 1. Borrelia anserina in turkey blood. This smear was made in the early stage of the disease. × 1,200.

Manual of Determinative Bacteriology lists them all as synonyms of *Borrelia anserina*. From cross-infection experiments by many authors this seems the best solution, although it is possible that different strains occur, as suggested by Marchoux and Salimbeni (1903), Sreenivasan and Sankaranarayan (1945), and others.

It should be emphasized that the spirochete referred to by Steinhaus and Hughes (1947) is not *Borrelia anserina*. The form described by them is smaller and more tightly coiled, and was found in eggs infected with chicken liver tissue. It was nonpathogenic for chickens, guinea pigs, and mice.

Borrelia anserina is not easily cultivated *in vitro*. El-Dardiry (1945) reported fairly successful results by the use of rabbit serum and Tyrode's solution. He believes serial passage in chickens to be the most practical method of maintaining

the spirochete. Hasson (1946) reported the use of egg albumin and serum in meat extract broth. Sreenivasan and Sankaranarayan (1945) found that infected citrated blood could be stored at 0 C for 3 weeks and then inoculated into chickens. Many workers prefer to maintain the spirochetes in the tick *Argas persicus*. In an area where the disease is not yet endemic, one hesitates to use this method. Knowles, Das Gupta, and Basu (1932) were able to grow the spirochetes in embryonated, but not in infertile, eggs. They quote others who have had the same results. In most cases the infection is sufficiently acute to kill the embryos before they hatch. We have had similar results with both

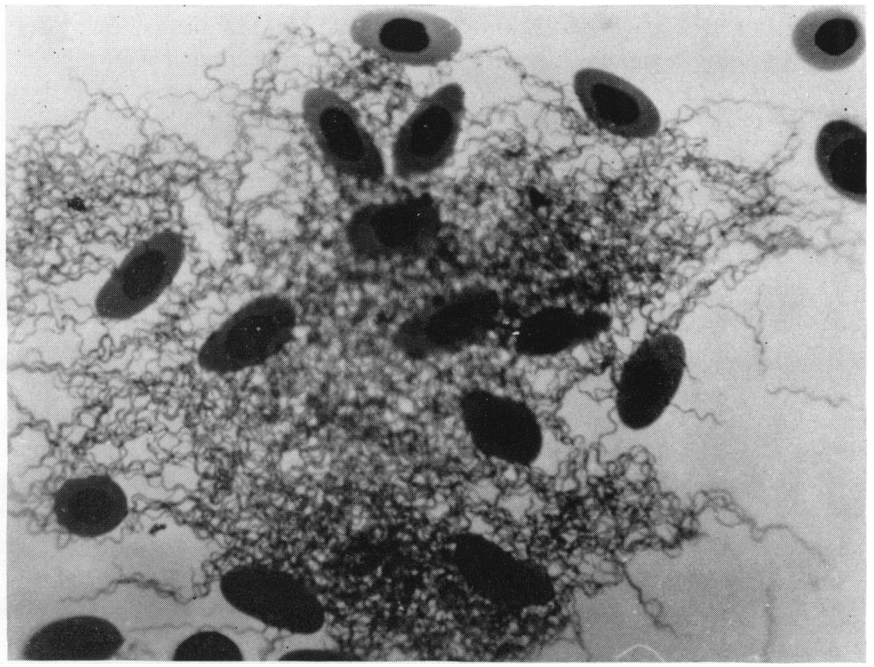

Figure 2. Borrelia anserina; showing clumping in the late stage of the disease. × 1,200.

chicken and turkey eggs. Attempts were made by us to lyophilize infected blood, but the spirochetes did not remain viable; at 20 F blood remained infective for 8 days.

In our laboratory we have maintained two strains by serial passage through chicks every 5 days. It is of interest to note that when we first acquired the organism from a subacute outbreak in turkeys it killed 35 per cent of the first 63 chicks used over a period of 6 weeks. After 2 years' passage in chicks it kills about 85 per cent. The greatest number of days on which we have found the blood of a chick to contain spirochetes is 17, the average is 7 days.

EPIDEMIOLOGY

Since its original report from the Caucasus, avian spirochetosis has been reported as a major cause of mortality in birds in North and South Africa, India,

Syria, Palestine, Turkey, the Balkan states, Greece, most of continental Europe, Russia, Australia, Java, Brazil, and Argentina. Knowles, Das Gupta, and Basu (1932) and Lesbouyries (1941) give reviews of its distribution and report natural outbreaks in chickens, geese, turkeys, ducks, grouse, and canaries. The following authors mention its occurrence in turkeys but give no exact data: Mezinescu and Calinescu (1909), Romania; Yakimoff (1916), Russia; El-Dardiry (1945), Egypt. The following birds have been experimentally infected by various workers: crows, myna birds, cominos, capucins, turtle doves, pigeons, guinea fowl, partridges, magpies, larks, and sparrows. Pigeons and guinea fowl are rather resistant to the infection. Young rabbits have been infected by intraperitoneal inoculation (Levaditi and Lange, 1905), but the infection was transitory. Climbing perch, geckoes, frogs, toads, squirrels, guinea pigs, mice, dogs, monkeys, and mules resisted infection, showing that it is truly an avian type of spirochete.

The disease is most often transmitted by *Argas persicus* (adults, nymphs, and eggs). Knowles *et al.* (1932) found spirochetes throughout the tissues of the tick. They obtained a higher percentage of infection by feeding ticks to chickens than through the bite of the tick. There have been conflicting reports concerning transmission by *Ornithodorus* and *Ixodes* ticks. Apparently the ticks may harbor the infection for many days, but it is questionable whether they often transmit it to fowl. Hungerford and Hart (1937) report transmission by the mite *Dermanyssus gallinae*. Since both infected and healthy birds were kept in the same cage, it cannot be certain that the mite was the transmitting agent. Zuelzer (1936) states that mites retain the infection for only 1 or 2 days and can only be mechanical transmitters, not vectors. On the other hand, she offered convincing evidence that *Culex* mosquitoes may be true vectors. By use of the precipitin test she proved the presence of fowl's blood in the mosquitoes in an infected area. When the mosquitoes were allowed to bite uninfected fowl, the latter became infected. Kapur (1940) failed to obtain transmission with *Aedes* mosquitoes. He was able to obtain transmission by smearing infected blood on the breast and comb of chickens, as were also Sreenivasan and Sankaranarayan (1945). The latter workers were able to demonstrate transmission by cohabitation. No ticks, mites, or lice were present on the ranches involved in the California outbreaks.

In chickens the incubation period varies from 3 to 8 days. There is usually a marked increase in temperature to 110 F or higher. The blood sugar may drop to 90 mg per cent. The birds become listless and often assume a crouching position. There is a greenish diarrhea, and paralysis of the wings or legs may occur. At autopsy the spleen is mottled and may be enlarged to six times normal size. The liver often shows peripheral necrotic areas. Mortality is high in both young and adult birds.

THE DISEASE IN TURKEYS

Most of the experimental work was done with turkey strain I, which was isolated by Dr. H. A. Hoffman in 1945 from the first authentic outbreak of avian

spirochetosis in the United States (Hoffman, Jackson, and Rucker, 1946). The strain was given to the writers for further study to prove its identity as *Borrelia anserina*. Both chicks and turkeys were used in the first part of the work.

Routes of infection. It was found that, as in chicks, the intravenous, intramuscular, and subcutaneous routes were the three which, in the order given, gave the shortest incubation times and greatest mortality. It has also been possible to establish infection in 6-day-old poults by nasal, oral, and intraorbital routes. All poults died within 10 days. Three-week-old poults inoculated orally, rectally, intraorbitally, nasally, and subcutaneously with strain II showed 50 per cent mortality in the first four groups, 100 per cent in the subcutaneously inoculated group, and no infection by nasal inoculation. Five-week-old poults became infected in 48 hours following intraorbital and rectal inoculation but aborted the infection by the fourth day. Poults of the same age inoculated subcutaneously with the same strain died. Chicks have been infected by all the foregoing routes with the same strain. Both poults and chicks have also been infected by intraperitoneal inoculation of macerated spleen and liver tissue of infected birds.

Adult turkeys have been inoculated by oral, intravenous, intramuscular, and intraperitoneal routes. They showed a rise in temperature in 24 to 48 hours and reached a peak (sometimes 111 F) in 3 to 5 days. Spirochetes appeared in the blood in 24 to 72 hours (table 1). Fourteen- to 16-week-old turkeys were also infected by intramuscular, intravenous, and subcutaneous routes.

Course of the disease: Poults. Poults (1 to 3 weeks old) usually received intramuscular or subcutaneous inoculations of 0.1 to 0.2 ml of infected blood.

Spirochetes appeared in the blood in 24 to 48 hours.The mortality resulting from such inoculations was 90 to 100 per cent.

Half-grown turkeys (10 to 16 weeks of age). If inoculated subcutaneously a rise in temperature usually occurred within 24 hours with a peak on the third, fourth, or fifth day. Spirochetes seldom appeared in the blood before 48 hours. The symptoms observed in adult birds (listlessness, bile-stained droppings, and diarrhea) were also in this age group. The mortality ranged from 60 to 100 per cent.

Adult turkeys. Four 7-month-old turkeys (3 females and 1 male) were inoculated with 1 ml of citrated blood as follows: male, intraperitoneally; 2 females, intravenously; and 1 female, intramuscularly. The male showed a rise in temperature in 24 hours, and spirochetes first appeared in the blood in 48 hours. Definite symptoms appeared in 72 hours, when a peak of 109.2 F was reached. The bird died on the seventh day following inoculation; it had lost 1,000 grams (one-sixth of its original weight) in this time. Yellow urates and bile-stained feces were abundant, and there was partial paralysis of the legs and wings.

One intravenously inoculated bird showed a rise in temperature in 24 hours, with the appearance of spirochetes in 24 hours, and a peak of 110 F on the fifth day. Bile-stained droppings were present on the third day. By the seventh day, the temperature had returned to normal, but the bird was droopy and had a profuse diarrhea. Spirochetes were found in the blood for 4 days.

TABLE 1

Examples of the relation of body temperature to the presence of spirochetes in turkeys

BAND NO.	AGE	METHOD OF INOC.	HOURS AFTER INOCULATION													
			24		48		72		96		120		144		168	
			Temp.	Blood	Temp.	Blood	Temp.	Blood	Temp.	Blood	Temp.	Blood	Temp.	Blood	Temp.	Blood
	mo															
1617	7	Int'ven.	107.8	2+	108.8	4+	108.5	3+	109.5	3+	109.8	Neg.	106.6	Neg.	106.6	Neg.
1791	7	Int'ven.	108.0	Few	108.2	2+	109.4	3+	108.6	4+	110+	Neg.	108.6	Neg.	106.4	Neg.
1826	7	Int'mus.	105.2	Neg.	105.6	Neg.	108.4	Few	108.4	2+	108.6	4+	108.4	4+	107.0	Neg.
1795	7	Int'per.	107.8	Neg.	108.4	2+	109.4	4+	109.2	5+	107	3+	108.1	3+	Died on 7th day	
904	3	Int'ven.		NE*	109.2	3+	109.2	4+	Dead							
901	3	Int'ven.		NE	109.4	3+	110.4	4+	107.4	5+	Dead					
809	3	Int'ven.		NE	110	3+	110	3+	110	Neg.	109.6	Neg.	107	Neg.		
1583	3	Int'ven.		NE	110	3+	108.2	5+	Dead							
1576	3	Int'ven.		NE	108.4	3+	110.2	5+	107	4+	Dead					
916	3	Int'ven.		NE	109.4	4+	110	5+	107.6	5+	Dead					
505	3½	Subcutan.	107	Neg.	108	1+	109.6	2+	109.4	4+	109.8	Neg.	108.4	Neg.	107.8	Neg. Died on 10th day
508	3½	Subcutan.	107.8	Neg.	109	1+	109.6	3+	109.8	3+	107.2	3+	108.4	Few	108.0	2+ Died on 7th day
509	3½	Subcutan.	108.8	Neg.	109.2	2+	109.8	4+	Dead							
593	3½	Subcutan.	108.4	Neg.	109.8	1+	110	3+	108.6	4+	Dead					
598	3½	Subcutan.	109.4	Neg.	109.8	1+	109	3+	Dead							
597	3½	Subcutan.	108.0	Neg.	108.6	Neg.	110	2+	110	3+	108.4	3+	Dead			

* NE = not examined; no symptoms.

The other intravenously inoculated bird showed an increase in temperature and spirochetes in the blood in 24 hours and a peak of 109.8 F on the fifth day, with a loss in weight of 750 grams. The blood smears were negative on the fifth day and marked improvement was noted from the sixth day. Spirochetes were found in the blood for 4 days.

The intramuscularly inoculated bird showed a rise in temperature in 48 hours, with a peak of 109.4 F on the fourth day, and spirochetes in the blood on the third day. Spirochetes were found in the blood for 4 days. Symptoms were first obvious on the fourth day, and by the twelfth day the bird had lost one-fifth of its original weight of 11.6 kilograms. On the eleventh day the bird was listless and the hock joints were involved. At the end of 6 weeks it had regained most of its weight.

Two adult chickens were inoculated at this time with the same amount of blood. The one that was inoculated intravenously died, and the one inoculated intramuscularly recovered.

Autopsy findings: Poults. At autopsy the liver was swollen and often had petechial hemorrhages; the heart was usually normal. The spleen was pale, mottled, and always two to three times its normal size; the kidneys were often pale and swollen. The most pathognomonic finding is the appearance of the spleen.

Half-grown turkeys. At this age petechial hemorrhages were occasionally present in the crop. There was often fibrinous pericarditis with coronary and subepicardial petechiae. The liver was enlarged, congested, sometimes mottled, and with albuminous degeneration. Peripheral infarcts were occasionally present, but not as consistently as reported for chickens. The spleen was almost always enlarged two to three times and mottled. The kidneys showed albuminous degeneration and ecchymoses; the ureters were usually full of yellowish urates. Catarrhal enteritis was generally present. Other lesions found occasionally included hemorrhagic duodenitis, ulceration at the junction of the proventriculus and gizzard, and petechiae in the omentum and serosa.

Adult turkeys. The breast muscles were often congested. The heart often showed fibrinous pericarditis. The livers were often studded with small abscesses, but peripheral necrotic areas were not so pronounced as in chickens. The spleen was almost always enlarged and showed the same characteristic mottling seen in other age groups (figure 3). The ovary was often congested or even hemorrhagic. The intestines showed a greenish, mucoid enteritis, marked congestion of the duodenum, and bile-stained contents, which in the rectum were whitish to yellowish green because of excessive urates.

HISTOPATHOLOGY

The characteristic structure of the spleen was not lost. The reticular cells were increased in number and size. They presented a foamy appearance due to the ingestion of lipoid material. The centers of the groups of reticular cells underwent hyalinization at times. Massive areas of hemorrhage were present (figure 4). This was the result of the rupture of the walls of veins and sinusoids.

The endothelial cells lining these structures were swollen and presented the foamy appearance seen in the primitive reticular cells. The diffuse lymphatic

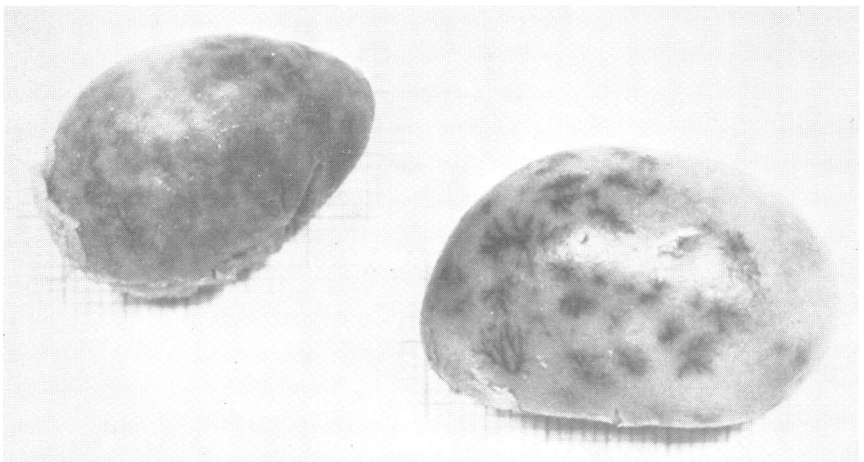

Figure 3. Spleens from adult infected turkeys that show the typical mottling and ecchymosis. Natural size.

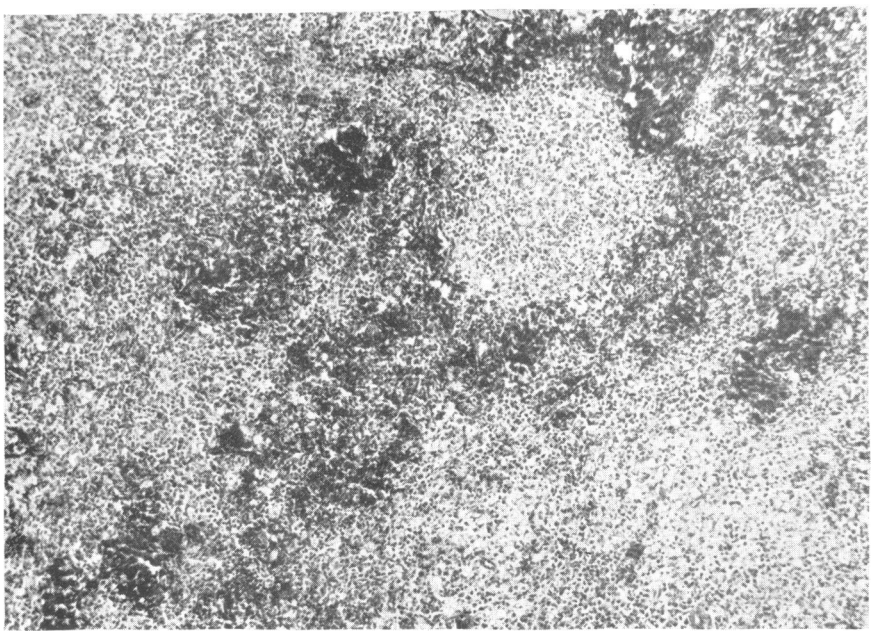

Figure 4. Section of spleen tissue showing massive areas of hemorrhage. × 150.

tissue was undergoing rapid growth. The cells consisted of young large- and medium-sized lymphocytes and hemocytoblasts. Numerous mitotic figures were present. In poults spirochetes occurred in foci throughout the spleen but did not appear to have been phagocytized by the reticular cells.

The liver showed congestion and an increase in the periportal lymphoid deposits. The cells of these deposits consisted of lymphocytes in all stages, hemocytoblasts, and large phagocytic cells with vacuolated cytoplasm. Various stages of erythropoesis were present. The endothelial cells lining the sinusoids were swollen and vacuolated. The parenchymal cells showed various degrees of fatty degeneration. Silver stains showed the majority of the spirochetes to be in the intercellular spaces and in the bile capillaries. Those within the hepatic cells underwent fragmentation or coiled upon themselves to form small rings.

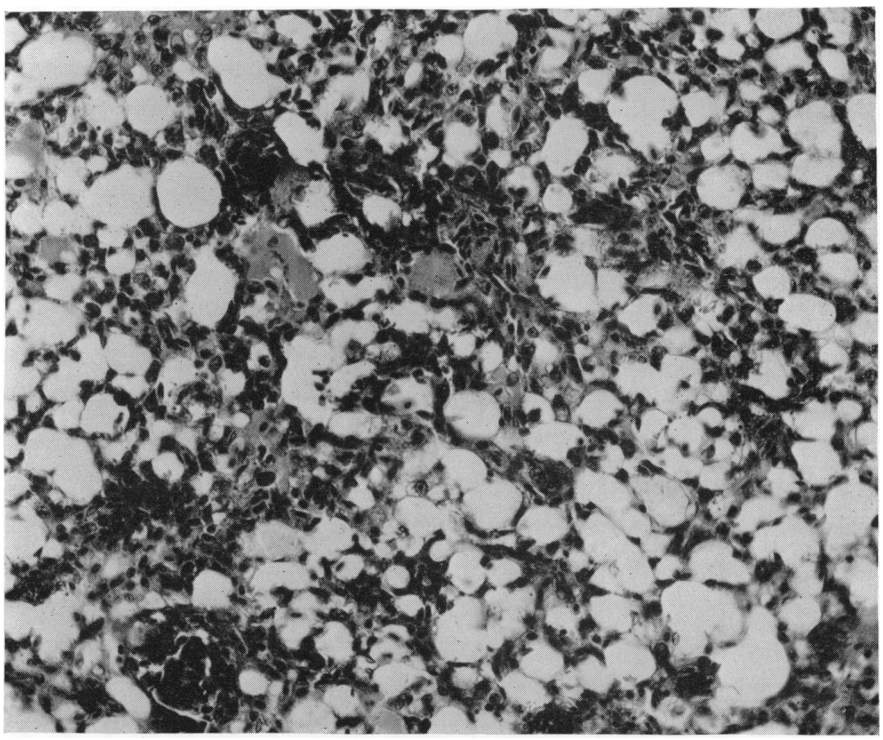

Figure 5. Section of lung tissue showing congestion and edema. × 350.

The lungs showed generalized congestion and edema (figure 5). The endothelial cells of the capillaries contained excess blood pigment. Some endothelial cells had hypertrophied so as to occlude the lumen of the vessel. Their cytoplasm was vacuolated, giving a foamy appearance. Small foci of hyaline necrosis were present throughout. The lymphoid nodules showed hemorrhage and hyperplasia.

The kidneys showed marked congestion and some hemorrhage. The glomeruli and convoluted tubules appeared normal. There was marked degeneration and desquamation with hyaline casts in the collecting tubules. The interstitial tissue showed lymphocytic infiltration. No spirochetes could be demonstrated in the kidney of the adults outside of the larger blood vessels. In poults spirochetes could be found in the intercellular spaces and in the lumens of the tubules.

The myocardial fibers presented a vacuolated appearance. In some areas this was so pronounced as to obliterate the cross striations. Spirochetes did not invade the heart tissue.

The seminiferous tubules appeared unaffected. The interstitial cells were large and filled with lipoid. The most striking feature was a prominent lymphocytic infiltration around the blood vessels. The ovary showed congestion and hemorrhage.

The intestines showed a marked degree of catarrhal enteritis. The lymphoid follicles in the submucosa were hypertrophied and there was a generalized lymphocytic infiltration of the submucosa. The tips of some of the villi were necrotic. The most severe injury occurred in the jejunum. The pancreas appeared normal except for slight vacuolization of the secreting cells.

The cerebrum and medulla showed a perivascular gliosis. The meninges showed a lymphocytic infiltration.

Survival of organism in tissues. Poults and chicks that had succumbed to the disease were stored at 32 F for varying periods of time and then thawed and autopsied. The spleen, liver, and heart were macerated and injected intraperitoneally into young chicks and poults. Tissues stored at this temperature contained viable, infective spirochetes for 31 days. Blood stored at 20 F for 8 days was still infective.

Cross-infection studies. Two adult silver pheasants were inoculated with 0.2 ml of infected blood, one intramuscularly and one intravenously. Both became infected and died 5 to 6 days after inoculation. Both showed lesions typical of spirochetosis in chickens and turkeys. Two lambs, two adult rabbits, three rats, and three fence lizards were inoculated, but none became infected.

IMMUNITY AND CHEMOTHERAPY

In chickens the disease produces an immunity for at least 13 months (El-Dardiry, 1945). One adult turkey in our experiments that had recovered from the disease was reinoculated twice during 18 months and was immune both times. When tested by the agglutination test using both a formalized and a merthiolated antigen, it had a titer of 1:320. A hyperimmunized chicken showed the same titer strength. This was considerably higher than in naturally recovered birds.

Cross-immunity tests were conducted with the two strains, using reciprocal serum-organism combinations, as well as organisms alone as a control. The results indicate that strain I, which had been isolated in 1945, was slightly more antigenic than strain II, which was isolated in 1947. The strain I serum was obtained from a hyperimmunized turkey and gave better protection than that obtained from strain II from a naturally recovered bird. This would indicate a definite advantage in producing hyperimmunized sera when such methods of prophylaxis are used (El-Dardiry, 1945).

In some parts of the world vaccines are made from infected tissues and blood. Nobrega and Reis (1941) obtained good results with a vaccine made from embryonated chicken eggs inoculated on the twelfth day of incubation and harvested 5 days later. They suggest this as a more economical method of preparation.

Some of the turkey poults exposed orally, nasally, rectally, and intraorbitally to infected blood showed no organisms in their blood but proved resistant to subsequent challenge. Knowles *et al.* (1932) report the presence of spirochetes in wing papillae when they were absent in the larger wing veins.

Uninoculated control poults left in contact with the infected ones have sometimes also proved resistant to subsequent inoculation. The only explanation that occurs to us is that these birds may have eaten an immunizing dose of spirochetes shed by pen mates. This would tend to confirm the results of Sreenivasan and Sankaranarayan (1945).

Birds treated with penicillin or streptomycin were resistant to subsequent inoculation.

El-Dardiry (1945) summarized the results of drug treatment. In general many of the arsenicals (salvarsan, myosalvarsan, "spirocid," and atoxyl) gave satisfactory results. Arrhenal (sodium methylarsonate) was not effective. None of the sulfonamides tested by him (sulfanilamide, sulfathiazole, sulfapyridine, and sulfadiazine) gave any beneficial results. The lot of penicillin used by him was not effective, but there is reason to believe that the lot was defective. Nobrega and Bueno (1945) reported the successful use of penicillin for spirochetosis in adult chickens when given at the rate of 10,000 units in 5 doses at 3-hour intervals. In 1946 they found that although 5,000 units per kilogram was sufficient to cure spirochetosis in adult chickens, a total of 20,000 units per kilogram was not sufficient for baby chicks. The drug was administrated as before in 5 doses at 3-hour intervals. They believe this to be due to the fact that no antibodies were yet built up in the baby chicks.

Penicillin.[3] We have had successful results with the use of penicillin for this disease in turkeys, both in the experimentally produced cases and in one field outbreak. These trials are given below.

Experiment 1. Eighteen 3-month-old turkeys were inoculated intravenously with 0.2 ml infected blood. Forty-eight hours later all showed an increase in temperature and spirochetes in the blood stream. Twelve received 10,000 units of penicillin (Merck) intramuscularly and six were left as controls. Of the 12, six received an additional 10,000 units the next day. Five of the 6 control birds died, and only 1 of the 12 treated birds. This bird was in crisis at the time of the first treatment and should have been treated 24 hours earlier.

Experiment 2. The two purposes of this experiment with 3½-month-old turkeys were to (1) find out whether the calcium and sodium salts of penicillin were of equal value and (2) to find the effective dosage. There appeared to be no difference between the sodium and calcium salts. The birds receiving 20,000, 15,000, or 10,000 units responded to treatment, and blood smears were negative in 24 hours. Of the two birds receiving 5,000 units, one responded in 24 hours, but the other had a positive blood smear for 3 days, was sick for a week, and lost weight. Under field conditions it would probably not have survived. Both birds receiving 2,000 units as well as the three untreated controls died. It is obvious that 10,000 units is the minimum effective dose.

[3] The penicillin and streptomycin used in these experiments were furnished by Dr. D. F. Green, Merck and Company.

Experiment 3. Four of the nine 6-week-old turkeys received 10,000 units of penicillin 72 hours after inoculation. All four showed marked improvement in 24 hours and the blood smears were negative. The five controls all had spirochetes at this time and were sick for several days. Again under field conditions four of the five controls probably would not have survived. The results of the use of penicillin in the field will be given later in this paper.

Streptomycin. Heilman (1945) reported fairly successful use of streptomycin in *Borrelia novyi* infections in mice, although not so successful as with penicillin. The following experiment shows that about the same results were obtained in avian spirochetosis:

Four 1-month-old poults were inoculated subcutaneously. Two developed heavy infections and two a mild infection. All received 15,000 units (micrograms) of streptomycin (Merck) intramuscularly. One of the four died, and two of four untreated controls having the same degrees of infection died.

Of the arsenicals, only two were tested, neoarsphenamine and mapharsen.

Neoarsphenamine. A single intramuscular treatment of 10 mg per kilogram or 20 mg per kilogram freed 3½-month-old turkeys of spirochetes within 48 hours; the untreated controls all died.

Mapharsen. A single intramuscular treatment of 5 mg per kilogram was effective against the spirochetes in 24 hours. When given at the rate of 12.5 mg per kilogram in the drinking water for 40 hours, both infected birds died. An uninfected control in the same cage appeared unaffected by the treatment.

Since there has been some doubt as to whether the arsenicals eliminated the spirochetes from all tissues, spleens and brains from the treated birds were removed 1 week after treatment, macerated, and inoculated into chicks. All chicks remained negative except one that had received brain tissue from a bird treated with 5 mg per kilogram of mapharsen. It developed a typical case of the disease.

DESCRIPTION OF FIELD OUTBREAK, 1947 (STRAIN II)

In January, 1947, the second outbreak of avian spirochetosis in turkeys in California was diagnosed by us. This outbreak occurred on a ranch in the San Joaquin Valley, and was about 200 miles south of the original one.

The flock in which the organism was found consisted of 6,000 breeder turkeys and was one of four large groups of birds owned by one grower. All groups were within one mile of one another. No ticks, mites, or lice were found on the premises at any time. A culicine mosquito (*Culex tarsalis*) winters over in that area and is known to attack livestock. It is also known that migratory waterfowl had been present in a temporary pond near the turkeys 3 weeks before the outbreak occurred and had remained about a week. Cranes, herons, coots, pelicans, and ducks were recognized, and coots and herons were seen feeding in the turkey yards. Quail and pheasants had also been abundant in the area, but none were seen in the yards during the outbreak. Blackbirds also came in large numbers to feed with the turkeys each morning and evening.

Visits were made by at least one of us each week for a period of 3 months to

study this outbreak. Almost all birds that died were autopsied by us, and treatment was given under our supervision. During the first 16 days of the outbreak the morbidity was about 20 per cent and the mortality about 2 per cent.

The autopsy findings were similar to the most acute experimentally produced cases. The livers were often studded with minute abscesses, peripheral necrosis was an occasional but not a constant finding, the kidneys were swollen, and the spleens were enlarged and mottled. Fibrinous pericarditis and congestion of the ovaries were often found. A greenish-yellow mucoid enteritis was constant. The highest temperature found was 109.4 F, with 108 to 108.4 F more common. Some infected birds sat in a penguinlike position; others became wholly or partially paralyzed. The temperatures of normal birds in the same flock varied from 103.8 F to 106.4 F, with 104 to 105 F as most common.

Treatment with penicillin was started January 29. At this time the disease was confined almost entirely to the hens. Each morning and evening the attendants picked out all birds that appeared subnormal. These were given 10,000 units of penicillin intramuscularly and placed in a separate "hospital" pen. When they had recovered, they were put back with the main flock. Since the legs had been marked with yellow paint, it was possible to tell whether the birds relapsed after treatment. Very few treated birds did relapse. Samples taken on a limited number of birds indicated that about 80 per cent of the birds became negative within 18 hours after treatment.

Since blackbirds were present in large numbers, blood smears were made from 125 of them taken on the infected ranches, but no spirochetes were present. Zuelzer (1936) reported transmission of spirochetes by culicine mosquitoes. As stated before, there were large numbers of mosquitoes present in this area, and circumstantial evidence points to the possibility of the introduction of infection by them. Bile-stained droppings from infected birds from this outbreak were fed to poults kept under laboratory conditions. The birds developed infection after 5 days.

By March 13 (6 weeks after the original diagnosis had been made) spirochetes were also found in two of the other flocks. The morbidity (over a 3-month period) in one of them was 381 per 3,600 or 11 per cent; the mortality was only 1 bird out of 2,400. The lower mordibity and mortality in these two flocks was perhaps due to awareness of the danger of transmission, and a diagnosis was made almost as soon as infection appeared, with immediate instigation of treatment.

Between 40,000 and 50,000 poults hatched from this flock were available to us for observation, as well as hatching records. At no time was there evidence of egg transmission of the spirochete, nor was hatchability lowered. There has been no recurrence of the disease on this ranch to date (September, 1948).

DISCUSSION

To clarify the use of the generic name, *Borrelia*, as used in the 1948 edition of *Bergey's Manual of Determinative Bacteriology*, a brief discussion is included here. The order *Spirochaetales* includes flexuous spiral forms with at least one complete

turn and is divided into the two families, *Spirochaetaceae* and *Treponemataceae*. The latter includes the forms having no definite protoplasmic structure. Of the three genera in this family, *Borrelia* includes the forms that stain readily with aniline dyes as contrasted to *Treponema* and *Leptospira*, which stain with difficulty except with Giemsa or silver impregnation. The spirochete from fowl stains readily with ordinary aniline dyes and does not have a terminal hook, and therefore falls in the genus *Borrelia*.

The form described by Steinhaus and Hughes (1947) is smaller, more tightly coiled, is referred to by them as often being "mere granules," and caused no mortality in chicks. Through the courtesy of Dr. Steinhaus we examined some of his preparations and found them to be very different from the spirochete discussed by us. It reminds one of the spirochetes described from chickens by Harris (1930). She gives an excellent historical review and then describes three types found in the ceca of chickens. Penetration of the intestinal epithelium was demonstrated, and spirochetes were also found in sections of the kidney of one bird. It seems reasonable to suggest that, since eggs are often penetrated by organisms of the intestinal tract, these spirochetes might occasionally penetrate and multiply within the egg. If penetration of the kidney is possible, penetration of the liver is likewise conceivable.

There is no doubt that the spirochete found in turkeys in California is *Borrelia anserina*. The lesions produced in chickens are identical with those described in detail by many authors. The morphology, increase of temperature, and incubation periods also correspond with those found elsewhere in chickens. The virulence of the strain I was increased by serial passage in chicks. Both laboratory and field observations lead us to agree with El-Dardiry (1945) that adult turkeys are more resistant than chickens. In breeding flocks it is not, as yet, of great economic importance in this country. If it should be introduced into poults, it would probably cause severe losses.

There is one important difference in the epidemiology of the outbreaks in California. No ticks, lice, nor mites were present at any time. It is possible that mosquitoes introduced the infection, but close observations in the field indicate that other methods of transmission, such as ingestion of blood by cannibalistic habits and ingestion of feces, play an important part in the dissemination of the disease.

In our laboratory transmission studies, in which equal amounts of inocula were used, no differences in sex susceptibility were observed. It was found that a much higher percentage of females than males were infected in the field outbreaks described in this paper. One could hypothesize that males might transmit the infection from female to female mechanically by the mating process. This is based on the fact that it was possible to infect poults by feeding them bile-stained feces from this outbreak.

A single dose of 10,000 units of penicillin has proved to be both an effective and an economical method of treatment for turkeys in this country. It is also one that growers find possible to administer under farm conditions. Sick birds are removed from the flock each morning, given an injection of penicillin, and placed in separate pens.

SUMMARY

Two strains of spirochetes, isolated from two outbreaks in turkeys in California, have been studied over a period of 2 years. They are the first strains of avian spirochetosis reported in North America. From morphological studies, as well as from animal inoculation experiments, they have been identified as *Borrelia anserina*.

Turkey poults have been infected by oral, intraorbital, nasal, rectal, intraperitoneal, intramuscular, and subcutaneous routes. They have also been infected by ingestion of bile-stained feces from a field outbreak.

The spirochetes will survive in the tissues of birds stored at 32 F for 31 days.

The enlarged, mottled spleen (2 to 4 times normal size) and the greenish mucoid enteritis are pathognomonic of this disease in turkeys, although a positive diagnosis depends on finding the organisms in the blood or tissues. Histopathological studies are reported in the text.

The mortality produced by these strains in young chickens and in turkey poults corresponds to that reported in chickens from other parts of the world. The mortality in older birds is lower, although morbidity may be high.

The morbidity in the field outbreak in adult turkeys reported in this paper varied in different pens from 5 to 20 per cent.

Cross-infection experiments proved chickens, turkeys, and pheasants to be susceptible, and rabbits, rats, lambs, and lizards to be resistant to infection.

No ticks, lice, or mites were found in either of the field outbreaks, nor have they been present at any time in the laboratory experiments. Mosquitoes were present at the time of the second field outbreak and may have introduced the infection from migratory birds. Blood smears from 125 blackbirds killed in infected yards were negative for spirochetes.

Agglutinins are produced by this spirochete.

Penicillin proved effective in a single dose of 10,000 units when given intramuscularly to infected birds from 6 weeks to 12 months of age. Streptomycin did not prove to be effective. Neoarsphenamine and mapharsen were no more effective than penicillin.

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